3. V. N. Galankin, Arkh. Patol., No. 11, 80 (1984).
4. I. V. Davydovskii, The Study of Infection [in Russian], Moscow (1956).
5. A. S. Kanayan, N. K. Permyakov, G. P. Titova, et al., Byull. Éksp. Biol. Med., No. 4, 447 (1988).
6. M. I. Kuzin, I. I. Kolker, and B. M. Kostyuchenok, Bacteriological Diagnosis of Wound Infection: Technical Recommendations [in Russian], Moscow (1984).
7. G. N. Kryzhanovaskii, V. D. Slepushkin, G. K. Zoloev, et al., Patol. Fiziol., No. 6, 47 (1987).
8. P. N. Napalkov, Khirurgiya, No. 5, 15 (1985).
9. E. L. Nasonov and V. A. Vinogradov, Byull. Vses. Kardiol. Nauch. Tsent., No. 1, 3 (1987).
10. A. V. Smol'yannikov and D. S. Sarkisov, Arkh. Anat., No. 3, 3 (1982).
11. E. S. Stanislavskii and I. I. Kolker, *Pseudomonas pyocyanea* Infection [in Russian], Moscow (1978).
12. V. K. Khugaeva, Byull. Éksp. Biol. Med., No. 3, 300 (1988).
13. G. N. Chistovich, The Pathogenesis of Staphylococcal Infection [in Russian], Leningrad (1961).
14. A. B. Shekhter, A. I. Solov'eva, S. E. Spevak, and M. I. Titov, Byull. Éksp. Biol. Med., No. 10, 487 (1988).

AUTORADIOGRAPHY OF PROTEIN SYNTHESIS AS A METHOD OF EVALUATING MORPHOLOGICAL AND FUNCTIONAL CHANGES IN BRAIN STRUCTURES

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UDC 616.831-009.1-092.9-07:616.831-008.939.6:577.122

KEY WORDS: autoradiography; protein synthesis; rat brain; L-3,4-dihydroxyphenylalanine; motor activity.

Since protein metabolism in the nervous system is ascribed a fundamental, if not decisive, role in its functional activity [6, 9], determination of protein synthesis at different levels of structural and functional organization of the brain, both under normal and under control experimental conditions, provides an indication of the particular features of the role of these structures in activity of the CNS.

The aim of this investigation was to assess the method of autoradiography of protein synthesis, combining macro- and microversions, for a comprehensive study of morphological and functional changes in brain structures at the level of anatomical formations and of individual types of neurons.

EXPERIMENTAL METHOD

As an indicator of functional changes in the CNS we chose an experimental model of reduced motor activity of animals, created by giving them an injection of L-3,4-dihydroxyphenylalanine (L-dopa) [1, 2, 8]. For this purpose, male Wistar rats weighing 160 ± 10 g were given an intraperitoneal injection of L-dopa in a dose of 100 mg/kg. A stable reduction of motor activity, characterized by an anxiety state, was observed after 3-4 weeks in the animals. Six rats which received L-dopa (three animals received L-dopa for 3 weeks and another three animals for 4 weeks) and three control rats, which received only injections of physiological saline for 4 weeks, were given an intraperitoneal injection of D,L-leucine-2-[$^3H_1$] (specific activity 8.8 mCi/mmole, from "Izotop," USSR) in a dose of 2 mCi/100 g body weight. The rats were decapitated 2.5 h after injection of the isotope under ether anesthesia, their brain was fixed in Carnoy's fluid and, after embedding

Laboratory of Cytochemistry, Brain Research Institute, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. S. Adrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 321-323, September, 1991. Original article submitted December 27, 1990.

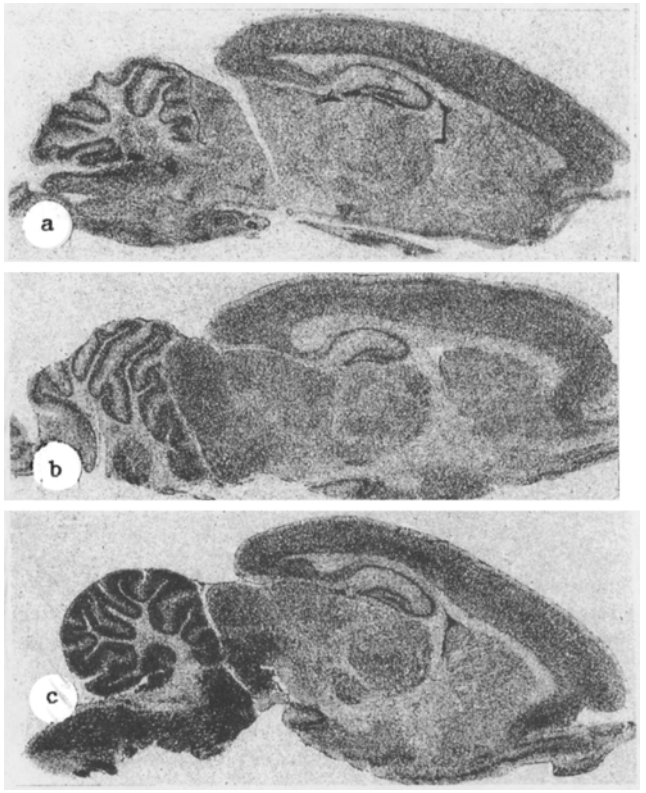

Fig. 1. Intensity of incorporation of ^{3}H-leucine at level of neuroanatomical formations of rat brain. Macroautoradiographs of sagittal sections through brain of control rats (a) and rats receiving injections of L-dopa in a dose of 100 mg/kg for 3 weeks (b) and 4 weeks (c). Photographic material recording radioactivity: LKB Ultrofilm 3 h; photomicrograph, magnification 6.

in paraffin wax, sagittal sections were cut to a thickness of 15 μ (left hemisphere of the whole brain) and frontal sections 5 min thick (sensomotor cortex and right caudate nucleus) were cut. To prepare macroautoradiographs sagittal brain sections were compared with LKB Ultrofilm ^{3}H photographic film. To prepare microautoradiographs, type M nuclear emulsion was applied to frontal sections. The emulsion layers were exposed at 4°C for 1 and 3 months respectively for LKB Ultrofilm ^{3}H and M emulsion, and then treated by the usual method [10]. The intensity of incorporation of [^{3}H]-leucine in macroautoradiographs was assessed from the degree of blackening of the emulsion layer, anatomical brain formations of the control and experimental rats being compared under a binocular loupe. Incorporation of the label at the cellular level was estimated quantitatively as described by the writer previously [7], using an automatic television image analysis system ("Leitz," Germany). For this purpose, the average density of grains of silver per unit area of cell structure was counted in the microautoradiographs, and the result was taken as an indicator of the intensity of incorporation of label into proteins of the neuron. Large pyramidal neurons in layer V — the projection-efferent type of cells — and small pyramidal neurons in layer III — cells of association type — in the sensomotor cortex, and in subcortical brain structures (caudate nucleus) — neurons of associative type — were studied. Between 150 and 300 cells were studied in a population of each type of neurons in the control and experimental rats. The results were subjected to statistical analysis by the Fisher—Student method.

EXPERIMENTAL RESULTS

A varied degree of incorporation of [^{3}H]-leucine was found in different anatomical formations. Maximal incorporation was found in the region of the choroid plexuses of the cerebral ventricles, in the gray matter of the cerebral and cerebellar cortex, the minimal quantity in fibrous (conducting) structures (Fig. 1a). Diminution of the motor activity of the experimental rats, caused by long-term (up to 4 weeks) administration of L-dopa, was characterized by an increase in the

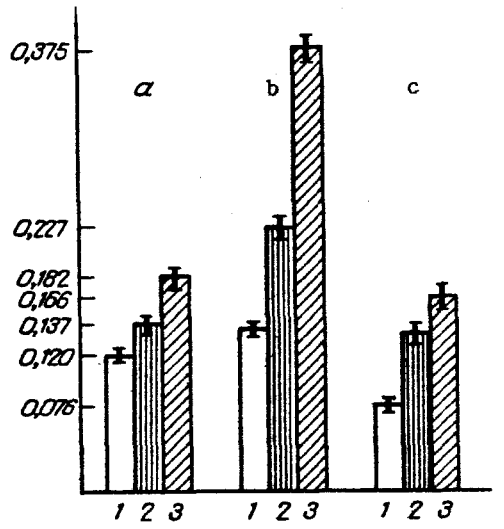

Fig. 2. Quantitative assessment of density of grains of silver in microautoradiographs of brain neurons of control rats and of rats receiving injections of L-dopa. Ordinate, density of grains of silver (in conventional units/μ^2 area of body of neuron). Neurons of layer III (a) and layer V (b) of sensomotor cortex and neurons of caudate nucleus (c) of rat brain. 1) Control, 2) rats receiving L-dopa for 3 weeks, and 3) for 4 weeks. Experimental conditions as in Fig. 1.

incorporation of label into proteins of the brain microstructures (Fig. 1b, c). However, differences of labeling in different anatomical formations were extremely great. For instance, in the motor nuclei of the medulla and cerebellum (the layer of granule cells and Purkinje cells) incorporation of the label increased strongly compared with the control, especially at the 4th week of the experiment, whereas in structures of layers of the sensomotor cortex and caudate nucleus, the increase was very small, so that no definite conclusion could be drawn regarding changes in protein synthesis. In this connection it was necessary to determine quantitatively the degree of incorporation of label in individual morphological and functional types of neurons in the sensomotor cortex and caudate nucleus. It was found (Fig. 2) that in the experimental rats not only was incorporation of ^{3}H-leucine increased in the nerve cells studied, but the intensity of incorporation also differed in different types of neurons. Compared with the control, the density of grains of silver per unit area of cell structure was increased at the 3rd week of the experiment by 19, 66, and 79%, and at the 4th week by 52, 174, and 118%, respectively, in neurons of layers III and V of the sensomotor cortex and in neurons of the caudate nucleus. The area of the bodies of the neurons in the experimental rats showed no significant change compared with the control.

Prolonged administration of large doses of L-dopa to intact rats, reducing their motor activity [1, 2, 8], led to various changes in protein synthesis in different brain structures. For instance, brain formations forming part of the higher levels of the motor analyzer, namely the sensomotor cortex and caudate nucleus, to judge by incorporation of ^{3}H-leucine in the macroautoradiographs, were apparently less involved in the reduction of the animals' motor activity than structures at lower levels of the motor analyzer, such as the motor nuclei in the medulla and the cerebellum, responsible for movement coordination and balance; these findings pointed to a wide degree of participation primarily of the deep brain formations controlling the animals' motor activity in the process. Meanwhile, the resolving power of macroautoradiography did not provide a solid basis for any definite conclusions regarding changes in protein synthesis when only small deviations in the intensity of incorporation of ^{3}H-leucine were found, as was observed in the sensomotor cortex and caudate nucleus. Counting grains of silver at the cellular level not only provided a solution to the problem of participation of the higher levels of the motor analyzer in these experiments, but also shed light on the degree of involvement of individual types of neurons in this process.

If it is recalled that changes in protein metabolism reflect functional readjustments in CNS activity [6, 9], changes in protein synthesis detected by autoradiography in the present experiment must be regarded as morphological and functional changes in brain structures. However, the nature of these changes remains unexplained. We know from the literature [3-5, 11] that injection of L-dopa into intact animals leads to hyperfunction of the dopamine system and to major changes in dopamine and neurotransmitter metabolism, which are bound to affect protein metabolism.

By means of macroautoradiography it is thus possible to survey the anatomical formations of the brain and to assess their involvement in the process in the course of a controlled experiment. Quantitative autoradiography at the cellular level not only permits a more accurate evaluation of the role of anatomical formations in this process, but also sheds light on the degree of involvement of individual morphological and functional types of neurons therein.

LITERATURE CITED

1. I. M. Aivazashvili, G. S. Iordanishvili, and M. I. Nikolaishvili, Neirokhimiya, No. 4, 141 (1985).
2. A. V. Val'dman and V. P. Poshivalov, Pharmacologic Regulation of Intraspecific Behavior [in Russian], Leningrad (1984).
3. L. M. Gershtein, A. V. Sergutina, and T. L. Chebotareva, Structural and Functional Bases of Integrative Brain Activity [in Russian], Moscow (1988), p. 103.
4. E. A. Gromova, Neurotransmitter Mechanisms of Memory and Learning [in Russian], Pushchino (1984), p. 3.
5. E. L. Dovedova, Zh. Nevropatol. Psikhiat., **88**, No. 7, 19 (1988).
6. O. N. Dolgov, A. B. Poletaev, and V. V. Sherstnev, Usp. Fiziol. Nauk, **11**, No. 3, 47 (1980).
7. R. M. Khudoerkov, Neirokhimiya, No. 1, 53 (1986).
8. K. M. Kantak and K. A. Meczek, Psychopharmacology, **96**, 468 (1988).
9. R. M. Pico and C. Gall, Brain Res., **497**, 387 (1989).
10. A. W. Rogers, Techniques of Autoradiography, 3rd. ed., Amsterdam (1979).
11. K. D. Wilner, J. Butler, W. E. Seifert, et al., Bichem. Pharmacol., **29**, 701 (1980).